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ELECTROPHORETIC COMPARISON OF *DENDROCTONUS PUNCTATUS* LECONTE AND *D. MICANS* (KUGELANN) (COLEOPTERA: SCOLYTIDAE)

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Abstract.—The taxonomic status of the American boreal spruce beetle, *Dendroctonus punctatus* LeConte, and its Eurasian sibling species, the European spruce beetle, *D. micans* (Kugelann), has been in doubt. The genetic relationship of adult *D. punctatus* from Montana, USA, and of *D. micans* from Belgium was examined by isozyme electrophoresis. Average heterozygosity and polymorphism was 0.075 and 37.5% for *D. punctatus* and 0.026 and 6.25% for *D. micans*, much lower than reported in other *Dendroctonus* species and which may result from the high degree of inbreeding that is characteristic of both species. Five of the 16 loci examined were fixed, or nearly fixed, for different alleles in the two species. Genetic identity between *D. punctatus* and *D. micans* was 0.693, lower than that reported between two other, uncontested, host-isolated sibling species, *D. ponderosae* Hopkins and *D. jeffreyi* Hopkins. Furthermore, the genetic distance between *D. punctatus* and *D. micans* was 0.366, similar to another distinct pair of host-isolated sibling species, *D. pseudotsugae* Hopkins and *D. simplex* LeConte. These results support recent morphological evidence in favor of retaining *D. punctatus* and *D. micans* as separate species.

Key Words.—Insecta, Scolytidae, *Dendroctonus punctatus*, *Dendroctonus micans*, isozyme electrophoresis

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The genus *Dendroctonus* Erichson is represented in North America by 17 species, including the boreal spruce beetle, *D. punctatus* LeConte, and in Eurasia by two species, including the European spruce beetle, *D. micans* (Kugelann) (Wood 1982, Wood & Bright 1992). Because the present center of diversity of *Dendroctonus* species is North America, the ancestor of *D. micans* is thought to have migrated from a spruce refugium in Alaska to Siberia via Beringia during the Wisconsinian glaciation and eventually reached Europe through intervening spruce forests. In recent times, *D. micans* has become of great economic importance where it has invaded new territory in Europe, particularly in exotic spruce plantations in France and England (Bevan & King 1983, Grégoire 1988). On the other hand, *D. punctatus* has so far caused little economic damage, being apparently at a competitive disadvantage in Nearctic boreal spruces to the economically important spruce beetle, *D. rufipennis* (Kirby) (Furniss 1995). Taxonomists have noted the anatomical similarity of *D. punctatus* and *D. micans* and have expressed uncertainty about their status as separate species (Wood 1963, 1982).

The biology of *D. micans* has been studied in Europe by Grégoire (1988); that of *D. punctatus* has been studied in western North America by Furniss (1995). Features that these two species share, differing from most others of the genus, are: a sex ratio strongly in favor of females; mating by siblings in the brood chamber prior to emergence (males never occur with females in egg galleries);

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and aggregation in the larval stage rather than as attacking adults (Grégoire 1983, Furniss 1995). Their main biological difference appears to be that *D. punctatus* has four larval instars, whereas *D. micans* is reported to have five larval instars (Furniss 1995). Morphologically, *D. punctatus* and *D. micans* have been found to differ in 10 discrete characters (Furniss and Johnson 1989, Furniss 1996). This paper presents results of isozyme electrophoresis that further support the validity of these two species.

Materials and Methods

Dendroctonus micans were collected as larvae at Wellin, Belgium, January 8, 1990 and sent to Moscow, Idaho, for rearing to the adult stage in 15 × 15 cm pieces of Norway spruce phloem (*Picea abies* (L.) Karst.) pressed between glass plates. *Dendroctonus punctatus* were F_1 progeny reared in Engelmann spruce logs (*P. engelmannii* Parry) (Furniss 1995). Their parents were collected from a white spruce hybrid (*P. glauca* (Moench.) Voss. × *engelmannii*) in Meagher Co., Montana in June 1989. Representative voucher specimens are deposited in the W. F. Barr Entomological Museum, University of Idaho.

Each adult was immersed in 1 cc of distilled water and macerated vigorously with pointed tweezers to develop a homogenate. Four filter paper wicks were soaked in each homogenate, wrapped individually in parafilm, and stored overnight at −18°C. Numbers of individuals tested were: *D. micans* = 58 female, 2 male; *D. punctatus* = 57 female, 2 male.

Electrophoretic techniques used were those of Stock et al. (1987). Gels were made from a 13% solution of hydrolyzed potato starch and the appropriate buffer. Wicks containing beetle homogenate were inserted into slots in each gel and subjected to electrophoresis. Gels were then cut horizontally and stained for different enzymes. Eleven enzyme systems were assayed (Table 1). Banding patterns were scored as homozygotes (appearing as a single band) or heterozygotes (appearing as multiple bands) for each gene locus resolved. Genotype frequencies for each population were recorded. Genetic data were analyzed using BIOSYS-1, a computer program for analysis of allelic variation (Swofford and Selander 1989).

Observed genotype frequencies were compared to values derived from random mating (Hardy-Weinberg expected proportions) using a chi-square test. Genetic diversity was estimated using Nei's (1978) unbiased estimate of average heterozygosity and polymorphism (%). A locus was considered polymorphic when the frequency of the most common allele was less than or equal to 0.99. The relationship between *D. punctatus* and *D. micans* was evaluated using Nei's (1978) genetic identity value, Nei's (1978) unbiased genetic distance value, and Rogers' (1972) similarity index.

Results and Discussion

Allele frequencies were calculated at 16 gene loci (Table 1). Major differences occurred between the two species at several loci. Six loci (Aat, Idh-2, Mdh-2, Me-1, Me-2, Mpi) were polymorphic in *D. punctatus* while only one (Me-2) was polymorphic in *D. micans*. As a result, average heterozygosity and polymorphism were much higher for *D. punctatus* (0.075 and 37.5%; respectively) than for *D. micans* (0.026 and 6.25%, respectively).

Table 1. Allele frequencies at 16 enzyme loci, percent polymorphism, average heterozygosity, and chi square comparisons of observed to expected (Hardy-Weinberg) numbers of each genotype for *D. punctatus* and *D. micans*.

Enzyme	Locus abbrev.	Allele	*D. punctatus*	*D. micans*
Aspartate aminotransferase	Aat	A	0.064	1.0
		B	0.936	0
		χ^2	45.8**	
Catalase	Ck	A	1.0	1.0
Esterase	Est-1	A	1.0	1.0
	Est-2	A	0	1.0
		B	1.0	0
	Est-3	A	1.0	1.0
Isocitrate dehydrogenase	Idh-1	A	1.0	1.0
	Idh-2	A	0.034	0
		B	0.966	1.0
		χ^2	78.0**	
Malate dehydrogenase	Mdh-1	A	1.0	1.0
	Mdh-2	A	0.964	0
		B	0.036	1.0
		χ^2	74.0**	
Malic enzyme	Me-1	A	0.034	0
		B	0.966	1.0
		χ^2	76.7**	
	Me-2	A	0.512	0.292
		B	0.488	0.708
		χ^2	0.7	6.3*
Phosphomannose isomerase	Mpi	A	0	1.0
		B	0.750	0
		C	0.250	0
		χ^2	20.8**	
Peptidase glycyl-leucine	Pep-gl	A	1.0	1.0
Peptidase leucyl-alanine	Pep-la	A	1.0	1.0
Glucose phosphate isomerase	Pgi	A	1.0	0
		B	0	1.0
Superoxide dismutase	Sod	A	1.0	1.0
% Polymorphism (0.99 criterion)			37.50	6.25
Av. heterozygosity			0.075	0.026

* = significant at 0.05 level, ** = 0.01 level.

In an earlier study (Stock et al. 1987), average heterozygosity and polymorphism of *D. micans* were 0.053 and 27%, respectively. However, the gene loci that were resolved and analyzed differed somewhat between their study and ours, perhaps contributing to the difference in values for *D. micans*. For example, the latter authors reported that locus Est-2 had six alleles; we observed only two. An additional possible source of the difference is foreign protein such as from a parasitic nematode (Higby and Stock, 1982). With that in mind, we had examined prior to maceration each of our specimens for ecto- and endoparasitic nematodes; *D. micans* contained none. If, however, the *D. micans* that were analyzed by Stock et al. (1987) had nematodes, that might explain the difference in heterozygosity and polymorphism of *D. micans* in the two studies. In any case, we tested paired sets of *D. micans* and *D. punctatus* simultaneously in the same gels, and we are

confident that the resultant values for heterozygosity and polymorphism truly reflect relative differences between the two species.

Dendroctonus punctatus and *D. micans* were much less genetically diverse than 10 other North American *Dendroctonus* species which have an average of 0.213 heterozygosity (range = 0.156–0.247) and 64% polymorphism (range = 50–72%) (Bentz and Stock 1986). Analysis of the deviations of alleles from Hardy-Weinberg equilibrium showed significantly less heterozygosity in *D. punctatus* than would be expected of random mating in a population (Table 1). This may be explained by the high degree of inbreeding in this species (Furniss 1995). The one locus that was polymorphic for *D. micans* showed more heterozygosity than expected. Selection may be favoring heterozygotes at this locus.

The fact that *D. micans* was less heterozygous and less polymorphic than *D. punctatus* may relate to the following. The immediate ancestor of *D. micans* must have migrated from an Alaskan glacial refugium. The particular genetic composition of individuals of this isolated population may have been subjected to intense selective pressure in its various, entirely new, host species and the differing climate as it extended thousands of miles eastward to Europe. On the other hand, during the time since *D. micans* migrated to Asia, its American ancestor has reunited with other population segments as its main host, *Picea glauca*, followed the retreating glaciers northward, eventually extending across the continent and throughout boreal North America.

Five loci (Aat, Est-2, Mdh-2, Mpi, Pgi) (Table 1) were fixed, or nearly fixed, for different alleles in the two species. Fixation of different alleles at one or more loci is characteristic of separate species or geographically separated non-interbreeding populations (Ayala and Powell 1972, Berlocher 1979, as cited by Higby and Stock 1982). Genetic similarity of *D. punctatus* and *D. micans* was 0.682. Conspecific populations of organisms commonly have similarity indices above 0.75 on a scale of 0-1 (Avise 1974, Ayala 1975).

The genetic identity index of *D. punctatus* and *D. micans* was 0.693. In comparison, the genetic identity index of two well-defined, host-isolated, sibling species, of Scolytidae, *D. ponderosae* Hopkins and *D. jeffreyi* Hopkins, was greater, being 0.83 (Higby and Stock 1982). That of conspecific populations of *D. ponderosae* from Utah in two different species of host trees (*Pinus contorta* Douglas and *P. ponderosa* Lawson) was 0.992–0.993 (Stock and Amman 1980) and the genetic identity index of *D. ponderosae* populations in Alberta in three pine hosts were above 0.978 (Langor and Spence 1991). In further comparison, the genetic identity of humans and chimpanzees is 0.680 and that of humans and Borneo orangutans is 0.707 (Bruce & Ayala 1979).

The genetic distance value for *D. punctatus* and *D. micans* was 0.366. This is somewhat similar to the genetic distance (0.305) of two uncontested sibling species, *D. pseudotsugae* Hopkins and *D. simplex* LeConte (Bentz and Stock 1986). No unique genetic difference was found between females and the few available males of *D. punctatus* and *D. micans*.

The genetic characters reported here supplement recent biological and morphological evidence (Furniss and Johnson 1989, Furniss 1995, Furniss 1996) in support of retaining *D. punctatus* and *D. micans* as separate species.

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